ELSEVIER

International Journal of Antimicrobial Agents 29 (2007) 451–455

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

Short communication

Antimicrobial resistance of *Arcobacter* and *Campylobacter* from broiler carcasses☆

Insook Son [a,b], Mark D. Englen [a,*], Mark E. Berrang [a], Paula J. Fedorka-Cray [a], Mark A. Harrison [b]

[a] *Bacterial Epidemiology and Antimicrobial Resistance Research Unit, US Department of Agriculture, Agricultural Research Service, Richard Russell Research Center, 950 College Station Road, Athens, GA 30605-2720, USA*

[b] *Department of Food Science and Technology, University of Georgia, Athens, GA 30602, USA*

Received 25 September 2006; accepted 30 October 2006

Abstract

The antimicrobial resistance of *Arcobacter* ($n = 174$) and *Campylobacter* ($n = 215$) isolated from broiler carcasses in a US poultry processing plant was examined. For *Arcobacter*, 93.7% ($n = 163$) were resistant to one or more antimicrobials and 71.8% ($n = 125$) were resistant to two or more antimicrobials. For *Campylobacter*, 99.5% ($n = 214$) were resistant to one or more antimicrobials and 28.4% ($n = 61$) were resistant to two or more antimicrobials. *Arcobacter butzleri* isolates were particularly resistant to clindamycin (90%; $n = 126$), azithromycin (81.4%; $n = 114$) and nalidixic acid (23.6%; $n = 33$). Resistance to tetracycline was very high in *Campylobacter jejuni* (99.5%) and *Campylobacter coli* (96.3%). Our results demonstrate substantial resistance in *Arcobacter* and *Campylobacter* to common antimicrobial agents.
Published by Elsevier B.V. on behalf of International Society of Chemotherapy.

Keywords: *Arcobacter*; *Campylobacter*; Broiler chickens; Antimicrobial resistance

1. Introduction

Arcobacters, previously classified as aerotolerant *Campylobacter*, are Gram-negative, non-spore-forming, microaerophilic or helical cells that are motile by means of polar flagella and exhibit a corkscrew movement [1]. *Arcobacter butzleri* is regarded as the primary human pathogen; *Arcobacter cryaerophilus* (subgroups 1A and 1B) is associated with human diarrhoeal illness and bacteraemia and with reproduction abnormalities in farm animals [2]. *Arcobacter skirrowii* has been reported in farm animals and on broiler carcasses. However, the pathogenic role of *Arcobacter* in human disease is still unclear.

Infection with *Campylobacter* is recognised as a leading cause of human enteritis worldwide, and *Campylobacter jejuni* and *Campylobacter coli* are most often associated with human infections [3]. Most patients with *Campylobacter* infections have a self-limited illness and do not require antimicrobial drugs except in cases with severe or prolonged symptoms or in immunocompromised patients [4]. When antimicrobial drugs are recommended for treatment, erythromycin or a fluoroquinolone such as ciprofloxacin are frequently the drugs of choice. However, the use of antimicrobial agents in food animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, including antimicrobial-resistant *Campylobacter* [5].

Among foods of animal origin, the occurrence of *Campylobacter* is much higher in poultry than in pork or beef [6]. *Arcobacter butzleri* has also often been identified on poultry products [2]. Many reports of antimicrobial resistance in *Campylobacter* isolated from poultry and other food animal sources have been published [5]. However, reports on antimicrobial resistance patterns in *Arcobacter* spp. isolated from

☆ Note: The mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

* Corresponding author. Tel.: +1 706 546 3066; fax: +1 706 546 3066.
E-mail address: menglen@ars.usda.gov (M.D. Englen).

0924-8579/$ – see front matter. Published by Elsevier B.V. on behalf of International Society of Chemotherapy.
doi:10.1016/j.ijantimicag.2006.10.016

poultry are lacking. Furthermore, there are no internationally accepted criteria for breakpoints and susceptibility testing for *Arcobacter* and *Campylobacter*, and validated reference strains for quality control have not been established.

In this study, the antimicrobial resistance patterns of *Arcobacter* and *Campylobacter* isolated from broiler carcasses during processing were determined using a broth microdilution test method and the results were compared.

2. Materials and methods

2.1. Sample collection and isolation of Arcobacter and Campylobacter

Broiler carcasses were collected on five visits to a commercial poultry-processing plant between August and October 2004 as previously reported [7] from three sites along the processing line: pre-scalding, pre-chilling and post-chilling. A total of 125 pre-scald carcass samples, 75 pre-chill samples and 125 post-chill samples were collected. Bacterial isolation was begun within 1 h of sample collection.

Both direct plating and sample enrichment methods were used for *Arcobacter* and *Campylobacter* isolation [7]. Presumptive identification was performed by microscopic examination of wet mounts of colonies using phase contrast optics. Isolates were stored at –70 °C.

2.2. Identification of Arcobacter and Campylobacter species

A modified multiplex polymerase chain reaction (PCR) for *Arcobacter* [8] was used for species identification, as described previously [7]. Reference strains of *Arcobacter*, including *A. butzleri* (ATCC 49616), *A. cryaerophilius* 1A (ATCC 43158), *A. cryaerophilus* 1B (ATCC 49615) and *A. skirrowii* (ATCC 51132), were used as controls. Reference strains and all presumptive *Arcobacter* isolates were cultured on *Brucella* agar (Hardy Diagnostics, Santa Maria, CA) supplemented with 5% (v/v) lysed horse blood (Lampire Biological, Pipersville, PA) at 25 °C for 48 h under ambient atmosphere.

Identification of *C. coli* and *C. jejuni* was determined using the BAX® PCR assay (Dupont Qualicon, Wilmington, DE) as described previously [9]. Reference strains for *Campylobacter* included *C. coli* (ATCC 33559) and *C. jejuni* (ATCC 33560).

2.3. Antimicrobial susceptibility testing

Susceptibility testing of *Arcobacter* and *Campylobacter* isolates was conducted using the protocol established for the U.S. National Antimicrobial Resistance Monitoring System (NARMS) for Enteric Bacteria. The custom-designed *Campylobacter* panel, providing serial dilutions of each antimicrobial, and a Sensititre® semiautomated system (TREK Diagnostic Systems Inc., Cleveland, OH) were used according to manufacturer's directions both for *Arcobacter* and *Campylobacter*. The frozen bacterial strains were subcultured on *Brucella* agar supplemented with 5% (v/v) lysed horse blood. *Arcobacter* strains were incubated for 48 h at 25 °C aerobically and *Campylobacter* strains were incubated microaerobically for 48 h at 42 °C. Colonies of *Arcobacter* and *Campylobacter* were suspended in Mueller–Hinton broth (TREK Diagnostic Systems Inc.) until the turbidity of the suspensions was adjusted to match that of a 0.5 McFarland standard. One hundred microlitres of the 0.5 McFarland suspension were transferred into 11 mL of Mueller–Hinton broth containing laked horse blood (TREK Diagnostic Systems Inc.), which was then used to inoculate the 96-well panel to give a final concentration of 10^5 colony-forming units/mL. *Campylobacter* panels included a control well with no antimicrobial drug. All panels were incubated in anaerobe jars containing 5% O_2, 10% CO_2 and 85% N_2 at 37 °C. The incubation time was 72 h for *Arcobacter* strains and 48 h for *Campylobacter* strains. Quality control ATCC strains *C. jejuni* 33560 and *A. butzleri* 49616 were tested to confirm susceptibility to all the antimicrobials at each testing. The minimum inhibitory concentration (MIC) for each antimicrobial was read as the first panel well in which no growth was visible. The antimicrobials tested and the resistance breakpoints (MICs) were: azithromycin, ≥2 μg/mL; ciprofloxacin, ≥4 μg/mL; clindamycin, ≥4 μg/mL; erythromycin, ≥32 μg/mL; gentamicin, ≥16 μg/mL; nalidixic acid, ≥32 μg/mL; and tetracycline, ≥16 μg/mL. The MICs of erythromycin, ciprofloxacin and tetracycline were classified as susceptible or resistant according to guidelines published by the Clinical and Laboratory Standards Institute for broth microdilution susceptibility testing. The MICs for the resistance breakpoints of the other antimicrobials were those used by NARMS as reported in the US Centers for Disease Control NARMS 2003 Annual Report (http://www.cdc.gov/narms/annual/2003/NARMS2003AnnualReport.pdf).

2.4. Data analysis

Differences in resistance to antimicrobials by species were analysed using the Wald χ^2 test by logistic regression model in the SAS statistical program (SAS Institute, Cary, NC). Differences were considered statistically significant at $P \leq 0.05$.

3. Results

3.1. Antimicrobial resistance

Most of the 174 *Arcobacter* isolates (93.7%; $n = 163$) were resistant to one or more antimicrobial agents. The percentages by species of *Arcobacter* isolates resistant to the individual antimicrobials are shown in Table 1. The highest prevalence of resistance among all *Arcobacter* isolates was to clindamycin (88.5%; $n = 154$), followed by azithromycin

Table 1
Percentage of *Arcobacter* and *Campylobacter* isolates resistant to various antimicrobials

Antimicrobial	*Arcobacter* spp.				*Campylobacter* spp.		
	A. butzleri ($n=140$)	*A. cryaerophilus* 1A ($n=4$)	*A. cryaerophilus* 1B ($n=30$)	Total *Arcobacter* spp. ($n=174$)	*C. jejuni* ($n=188$)	*C. coli* ($n=27$)	Total *Campylobacter* spp. ($n=215$)
Azithromycin	81.4 ($n=114$)[a]	75 ($n=3$)[a]	13.3 ($n=4$)[a,b]	69.5 ($n=121$)	0	0	0
Ciprofloxacin	0	0	3.3 ($n=1$)	0.6 ($n=1$)	30.3 ($n=57$)[a]	3.7 ($n=1$)[b]	27.0 ($n=58$)
Clindamycin	90.0 ($n=126$)	100.0 ($n=4$)	80.0 ($n=24$)	88.5 ($n=154$)	1.6 ($n=3$)	0	1.4 ($n=3$)
Erythromycin	4.3 ($n=6$)	0	0	3.4 ($n=6$)	0	0	0
Gentamicin	0	0	0	0	0	0	0
Nalidixic acid	23.6 ($n=33$)	0	10.0 ($n=3$)	20.7 ($n=36$)	30.3 ($n=57$)[a]	3.7 ($n=1$)[b]	27.0 ($n=58$)
Tetracycline	0	0	0	0	99.5 ($n=187$)	96.3 ($n=26$)	99.1 ($n=213$)

[a,b] Values within rows with no common superscripts differ significantly ($P \leq 0.05$) by Wald χ^2 test.

(69.5%; $n=121$) and nalidixic acid (20.7%; $n=36$). The percentage of *Arcobacter* isolates resistant to ciprofloxacin and erythromycin was very low, and all *Arcobacter* isolates were susceptible to gentamicin and tetracycline. Resistance in the *A. butzleri* group to azithromycin was much higher than in the *A. cryaerophilus* 1B group (81.4% versus 13.3%), whilst resistance to nalidixic acid in the *A. butzleri* group was not significantly different from the *A. cryaerophilus* 1B strains (23.6% versus 10.0%). Resistance to erythromycin was observed only in *A. butzleri* at a low level (4.3%). Resistance to ciprofloxacin was only found in one *A. cryaerophilus* 1B strain (Table 1). The site of isolate collection (pre-scald, pre-chill and post-chill) did not affect the observed antimicrobial resistance patterns (data not shown).

For *Campylobacter*, 99.5% ($n=214$) of the 215 *Campylobacter* isolates were resistant to one or more antimicrobial agent. The percentages by species of *Campylobacter* isolates resistant to the individual antimicrobials are shown in Table 1. Tetracycline resistance was the highest at 99.1% ($n=213$) for all *Campylobacter* isolates. The next most common resistance was to ciprofloxacin and nalidixic acid (27.0%; $n=58$). The percentages of resistance among the *C. jejuni* isolates to ciprofloxacin and nalidixic acid were more than eight times higher compared with the *C. coli* group (30.3% versus 3.7% for both antimicrobials). However, resistance to tetracycline for *C. jejuni* and *C. coli* was not significantly different (99.5% versus 96.3%). Resistance to clindamycin was found only in *C. jejuni* (1.6%; $n=3$). All *Campylobacter* strains were susceptible to azithromycin, erythromycin and gentamicin (Table 1).

3.2. Multiple resistances

Of the 174 *Arcobacter* isolates tested, 71.8% ($n=125$) were resistant to two or more antimicrobials. A significantly higher percentage of the *A. butzleri* (82.9%; 116/140) and *A. cryaerophilus* 1A strains (75%; 3/4) belonged to this group compared with *A. cryaerophilus* 1B (20%; 6/30). The majority of these 125 *Arcobacter* strains (76%; $n=95$) were resistant to two antimicrobials (Table 2). Of the 95 *Arcobacter* isolates resistant to two antimicrobials, 83 (77 *A. butzleri*, 3 *A. cryaerophilus* 1A and 3 *A. cryaerophilus* 1B) combined resistance to azithromycin/clindamycin. Other double resistances were found for azithromycin/erythromycin (5 *A. butzleri*) and clindamycin/nalidixic acid (5 *A. butzleri* and 2 *A. cryaerophilus* 1B). Resistance to three antimicrobials for *Arcobacter* was found in 24% ($n=30$) of the 125 multiresistant isolates (or 17.2% of all *Arcobacter* isolates) (Table 2). The most frequently observed combination was azithromycin/clindamycin/nalidixic acid, found only in *A. butzleri* strains (93.3%; 28/30) (Table 2). The remaining two patterns of triple resistance contained a single *Arcobacter* isolate each (Table 2).

For the 215 *Campylobacter* isolates, 28.4% ($n=61$) were resistant to two or more antimicrobials (Table 2). The highest percentage (93.4%) among these 61 isolates was the

Table 2
Resistance patterns of *Arcobacter* and *Campylobacter* isolates resistant to two or more antimicrobials

No. of resistances	Resistance pattern	No. of isolates with resistance pattern				
		A. butzleri ($n=116$)	*A. cryaerophilus* 1A ($n=3$)	*A. cryaerophilus* 1B ($n=6$)	*C. jejuni* ($n=60$)	*C. coli* ($n=1$)
2	AZM, CLI	77	3	3	–	–
2	AZM, ERY	5	–	–	–	–
2	CIP, NAL	–	–	–	–	1
2	CLI, NAL	5	–	2	–	–
2	CLI, TET	–	–	–	3	–
3	AZM, CLI, ERY	1	–	–	–	–
3	AZM, CLI, NAL	28	–	–	–	–
3	CIP, CLI, NAL	–	–	1	–	–
3	CIP, NAL, TET	–	–	–	57	–

AZM, azithromycin; CLI, clindamycin; ERY, erythromycin; CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline.

combination of resistance to ciprofloxacin/nalidixic acid/tetracycline ($n=57$ *C. jejuni*). Resistance to ciprofloxacin/nalidixic acid was found in one *C. coli* strain and resistance to clindamycin/tetracycline was observed in three *C. jejuni* strains.

4. Discussion

In this study, the antimicrobial resistance patterns of *Arcobacter* and *Campylobacter* isolated from broiler carcasses in a poultry-processing plant were examined and compared. To accomplish this, the NARMS criteria for *Campylobacter* were adopted to categorise the isolates of *Arcobacter* as susceptible or resistant because there are currently no available data that can be used for the interpretation of broth microdilution susceptibility testing for *Arcobacter*.

The total percentage of *Arcobacter* isolates (93.7%; 163/174) resistant to one or more antimicrobials was only slightly lower than that of *Campylobacter* isolates (99.5%; 214/215). However, the *Arcobacter* isolates were more diverse than the *Campylobacter* isolates. Previous work in our laboratory had shown that many of the *Campylobacter* strains were indistinguishable by pulsed-field gel electrophoresis [7]. Only 0.5% (1/188) of the *C. jejuni* and none of the *C. coli* strains were susceptible to all antimicrobials, whilst 4.3% (6/140) of *A. butzleri* and 16.7% (5/30) of *A. cryaerophilus* 1B were pan-susceptible.

A high level of resistance in *Arcobacter* species was found to azithromycin, although resistance to this macrolide was much higher in *A. butzleri* (81.4%) than in *A. cryaerophilus* 1B (13.3%). Indeed, resistance to azithromycin in *A. butzleri* strains was the second highest among the antimicrobials tested; the highest resistance for all *Arcobacter* species tested in this study was to clindamycin (88.5%), a drug recommended as an alternative treatment for *Campylobacter* gastroenteritis in humans [10]. Other authors have also reported a high level of resistance to clindamycin in *A. butzleri* from human and animal isolates [11]. In contrast, resistance to erythromycin was very low in *A. butzleri*, whilst all the *A. cryaerophilus* 1A and 1B isolates were susceptible to erythromycin. Compared with the *Arcobacter* strains, all *Campylobacter* strains were susceptible to macrolide/lincosamide agents. This indicates that macrolides/lincosamides can still be considered drugs of choice for treating *Campylobacter* infections, but the decision to use azithromycin and clindamycin for treating *Arcobacter* infection requires more data from other food animal sources and human isolates.

Kassenborg et al. [12] reported that in the USA, poultry is an important source of domestically acquired fluoroquinolone-resistant *Campylobacter* infection. However, a comparison of resistance rates to fluoroquinolones in the USA since 2000 shows little change between human and poultry *Campylobacter* isolates (http://www.fda.gov/cvm/narms_pg.html). In contrast, data for fluoroquinolone resistance in *Arcobacter* from animal or human disease are lacking. We found that all *A. butzleri* and *A. cryaerophilus* 1A were susceptible to ciprofloxacin, and only one *A. cryaerophilus* 1B was resistant to this agent. The *Campylobacter* strains had a higher overall resistance to ciprofloxacin (27%) compared with the *Arcobacter* strains, although *C. coli* resistance was only 3.7%. Resistance to nalidixic acid was similar both for *Arcobacter* (20.7%) and *Campylobacter* strains (27%). Fera et al. [11] reported that the fluoroquinolones levofloxacin, marbofloxacin, enrofloxacin and ciprofloxacin showed good activity against *A. butzleri* and *A. cryaerophilus* stains, whilst Atabay and Aydin [13] found that all strains of *A. butzleri* were susceptible to nalidixic acid.

All *Arcobacter* and *Campylobacter* strains included in our study were susceptible to the aminoglycoside gentamicin. Other authors [11] have also reported that *Arcobacter* was susceptible to aminoglycosides, including kanamycin, amikacin, gentamicin and streptomycin. *Campylobacter jejuni* and *C. coli* resistance to kanamycin and streptomycin has been well documented [5].

To our knowledge, tetracycline resistance in *Arcobacter* isolates from broiler chickens has not been reported, and all *Arcobacter* strains in the present study were susceptible to

tetracycline. This suggests that tetracycline may be useful for the treatment of *Arcobacter* infections in human and veterinary medicine, along with aminoglycosides. However, the level of resistance to tetracycline in *Campylobacter* species exceeded 99%. A similar high incidence of tetracycline resistance was reported in Taiwan in *C. jejuni* and *C. coli* from chicken products (83% and 90%, respectively) [14].

Most of the *A. butzleri* strains showing multidrug resistance had resistance to azithromycin/clindamycin (66.4%; 77/116). Accordingly, the predominant resistance pattern to three antimicrobials in *A. butzleri* was azithromycin/clindamycin/nalidixic acid, found in 96.6% (28/29) of these strains resistant to three antimicrobials and 93.3% (28/30) of all *Arcobacter* isolates in this category. In *Campylobacter*, resistance to three antimicrobials was found at a higher level than resistance to two antimicrobials and, among species, multiresistance was observed more than eight times as often in *C. jejuni* strains (31.9%; 60/188) compared with *C. coli* (3.7%; 1/27). Resistance to ciprofloxacin/nalidixic acid/tetracycline was by far the most common multiresistance pattern in the *C. jejuni* isolates (95%; 57/60).

In conclusion, *Arcobacter* and *Campylobacter* strains from a poultry-processing plant showed resistance to a relatively narrow range of antimicrobials. Moreover, the percentage of multiply resistant strains was much higher in *Arcobacter* than in *Campylobacter* strains. Long-term antimicrobial susceptibility surveillance for *Arcobacter* and *Campylobacter* isolates is needed to evaluate properly the effect of antimicrobial usage in food production animals.

Acknowledgments

The authors thank Mark N. Freeman and Scott R. Ladley of the USDA Bacterial Epidemiology and Antimicrobial Resistance Research Unit for assistance with sampling.

References

[1] Moore JE, Corcoran D, Dooley JS, et al. *Campylobacter*. Vet Res 2005;36:351–82.
[2] Lehner A, Tasara T, Stephan R. Relevant aspects of *Arcobacter* spp. as potential foodborne pathogen. Int J Food Microbiol 2005;102:127–35.
[3] Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. Emerg Infect Dis 1999;5:607–25.
[4] Ketley JM. Pathogenesis of enteric infection by *Campylobacter*. Microbiology 1997;143:5–21.
[5] Aarestrup FM, Engberg J. Antimicrobial resistance of thermophilic *Campylobacter*. Vet Res 2001;32:311–21.
[6] Jacobs-Reitsma WF. *Campylobacter* in the food supply. In: Nachamkin I, Blaser MJ, editors. *Campylobacter*. 2nd ed. Washington, DC: ASM Press; 2000.
[7] Son I, Englen MD, Berrang ME, Fedorka-Cray PJ, Harrison MA. Genetic diversity of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. J Food Prot 2006;69:1028–33.
[8] Kabeya H, Kobayashi Y, Maruyama S, Mikami T. One-step polymerase chain reaction-based typing of *Arcobacter* species. Int J Food Microbiol 2003;81:163–8.
[9] Englen MD, Fedorka-Cray PJ. Evaluation of a commercial diagnostic PCR for the identification of *Campylobacter jejuni* and *Campylobacter coli*. Lett Appl Microbiol 2002;35:353–6.
[10] Smith KE, Bender JB, Osterholm MT. Antimicrobial resistance in animals and relevance to human infections. In: Nachamkin I, Blaser MJ, editors. *Campylobacter*. 2nd ed. Washington, DC: ASM Press; 2000.
[11] Fera MT, Maugeri TL, Giannone M, et al. In vitro susceptibility of *Arcobacter butzleri* and *Arcobacter cryaerophilus* to different antimicrobial agents. Int J Antimicrob Agents 2003;21:488–91.
[12] Kassenborg HD, Smith KE, Vugia DJ, et al. Fluoroquinolone-resistant *Campylobacter* infections: eating poultry outside of the home and foreign travel are risk factors. Clin Infect Dis 2004;38(Suppl. 3):S279–84.
[13] Atabay HI, Aydin F. Susceptibility of *Arcobacter butzleri* isolates to 23 antimicrobial agents. Lett Appl Microbiol 2001;33:430–3.
[14] Li CC, Chiu CH, Wu JL, Huang YC, Lin TY. Antimicrobial susceptibilities of *Campylobacter jejuni* and *coli* by using E-test in Taiwan. Scand J Infect Dis 1998;30:39–42.